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Changes in pH differently affect the binding properties of histamine H_1 receptor antagonists

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Abstract

We investigated the effect of acidic pH, a condition that can be encountered during inflammation accompanying allergic reaction, on the binding properties of histamine H_1 receptor antagonists, including levocetirizine ((2-{4-[(*R*)-(4-chlorophenyl)(phenyl)methyl]piperazin-1-yl} ethoxy)acetic acid; Xyzal®), fexofenadine (*rac*-2-[4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl) piperidin-1-yl]butyl]phenyl]-2-methylpropionic acid hydrochloride; Allegra®) and desloratadine (8-Chloro-6,11-dihydro-11-(4-piperidylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine; Clarinex®). Lowering the pH from 7.4 to 5.8 decreased the affinity of [^{3}H]mepyramine for histamine H_1 receptors from 1.7 to 7.5 nM while the opposite was observed with [^{3}H]levocetirizine, whose affinity increased from 4.1 to 1.5 nM. Competition curves with [^{3}H]mepyramine indicated that decreasing the pH from 7.4 to 5.8 led to a 2- to 5-fold increase in the affinity of fexofenadine and levocetirizine, no change in affinity for desloratadine and a 5- to 10-fold decrease in affinity for mepyramine and histamine. Kinetic experiments showed that the increase in affinity of levocetirizine and, to a lesser extent, fexofenadine were totally attributable to a lower dissociation rate at acidic pH ($t_{1/2}$ increasing from 77 to 266 min and from 71 to 135 min, respectively). Although the affinity of desloratadine remained unchanged, lowering the pH caused a decrease in its dissociation rate ($t_{1/2}$ of 50 and 256 min at pH 7.5 and 5.8, respectively) accompanied by a concomitant 3.5-fold decrease in its association rate constant. The loss of affinity of mepyramine at acidic pH was driven by a decrease in its association rate constant. Interaction between the carboxylic moiety of levocetirizine and Lys^{191} is responsible for its slow dissociation rate from the receptor. We found that the magnitude of the pH effect on the dissociation rate of levocetirizine was maintained after mutating Lys^{191} into alanine, suggesting that a tighter interaction of levocetirizine with Lys^{191} at lower pH is not the cause of its even slower dissociation rate from the receptor. Although these changes may seem limited in amplitude, we show that they may have substantial effects on receptor occupancy in vivo.

Keywords: Histamine H_1 receptor; Antihistamine; Binding; Levocetirizine; Desloratadine; Fexofenadine

1. Introduction

Histamine H_1 receptor antagonists are particularly effective to treat allergic rhinoconjunctivitis and urticaria. They also display some efficacy in relieving seasonal asthma symptoms concomitant to allergic rhinitis (for review, see Simons, 2004) and cetirizine has been reported to delay the onset of asthma in children suffering from atopic dermatitis and being sensitised to grass pollen and/or house dust mite (Warner, 2001). One of the hallmarks of these pathological conditions is the influx of inflammatory cells to the scene of the allergic reaction. The high metabolic activity of such inflammatory cells along with the potential presence of infectious micro-organisms as seen for example during asthma exacerbations may lead to local acidosis. A drop in pH (from 0.5 log up to 2–3 log) in the exhaled breath condensate which contains aerosolized lung lining fluid has been described in asthma, chronic obstructive pulmonary disease and upper respiratory tract infection (Hunt, 2002). The skin pH is also well known to be acidic with values as low as 5 at the skin surface and still below 7 in the horny layer; although in atopic dermatitis and contact dermatitis the mean values reported are a little higher (5.3 to 5.5 instead of 5.0–5.1), they are still quite acidic (Eberlein-König et al., 2000; Rippke et al., 2002).

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Considering that inflammation causes an acidic microenvironment, Vermeulen et al. (2004) investigated the effects of lowering the pH to 6–6.5 on the reactivity of dendritic cells and the authors reported an increase in the endocytosis process of immature dendritic cells along with an up regulation of cell surface proteins involved in antigen presentation, making these cells more apt to respond to antigen in acidic conditions. Since antihistamines are widely prescribed in such pathological conditions, we decided to study the effects of acidic pH on their binding characteristics to human histamine H_1 receptors. Ligand binding to G protein-coupled receptors implicates ionic interactions with key amino acid residues localized in the binding pocket of the receptor and these interactions can be sensitive to the pH depending on the chemical nature of the functional groups that are involved. Hence, the affinity of the ligand will be pH dependent. This has been verified, for example, in the case of compounds binding to muscarinic receptors (Ehlert and Delen, 1990), thromboxane A_2 receptors (Theis et al., 1992) or $P2X_2$ purinergic ligand gated ion channel (King et al., 1997).

2. Materials and methods

2.1. Drugs and radioligands

Levocetirizine ((2-{4-[(*R*)-(4-chlorophenyl)(phenyl)methyl] piperazin-1-yl}ethoxy)acetic acid; Xyzal®), and desloratadine (8-Chloro-6,11-dihydro-11-(4-piperidylidene)-5*H*-benzo[5,6] cyclohepta[1,2-*b*]pyridine; Clarinex®) were synthesized at UCB S.A. (Braine l'Alleud, Belgium). Fexofenadine (*rac*-2-[4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl] butyl]phenyl]-2-methylpropionic acid hydrochloride; Allegra®), histamine, mepyramine, (+)chlorpheniramine and DNAse were purchased from Sigma-Aldrich (Bornem, Belgium). [Pyridinyl-5-^{3}H]pyrilamine ([^{3}H]mepyramine, 27 Ci/mmol) was purchased from Amersham Biosciences (Rosendaal, The Netherlands). [^{3}H]levocetirizine (27 Ci/mmol) was custom synthesized by CEA (Saclay, France). Ham's F-12, Dulbecco's phosphate-buffered saline (PBS), penicilline, streptomycine, foetal bovine serum and L-glutamine were bought from Cambrex (Verviers, Belgium). Geneticin was purchased from Invitrogen (Merelbeke, Belgium). All other reagents were of analytical grade and obtained from conventional commercial sources.

2.2. Cloning and site-directed mutagenesis

Cloning and stable expression of human wild-type and Lys^{191} mutant histamine H_1 receptors in Chinese hamster ovary (CHO) cells were performed as described in Moguilevsky et al. (1994) and Gillard et al. (2002).

2.3. Cell culture and membrane preparation

CHO cells were subcultured in Ham's F-12 medium containing 2 mM L-glutamine, 50 IU/ml penicilline, 50 μg/ml streptomycine, 400 μg/ml geneticin and supplemented with 10% foetal bovine serum. The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Confluent cells were detached by a 10-min incubation in PBS containing 1 mM EDTA. The cell suspension was centrifuged twice for 10 min at 1500×*g* (4 °C). The cell pellet was homogenized in a ice-cold 15 mM Tris–HCl buffer (pH 7.4) containing 1 mM EGTA, 0.3 mM EDTA and 2 mM $MgCl_2$. The homogenate was frozen in liquid nitrogen, thawed and incubated for 10 min at 25 °C with 10 IU/ml of DNAse before being centrifuged for 25 min at 40 000×*g* (4 °C). The resulting pellet was resuspended in a 50 mM Tris–HCl buffer (pH 7.4) containing 250 mM sucrose and stored, at a protein concentration of 3–4 mg/ml, in liquid nitrogen.

2.4. Equilibrium binding experiments

Histamine H_1 receptor binding assays were performed as described in Gillard et al. (2002) with few modifications. Briefly, membranes (15 to 50 μg of proteins) from CHO cells expressing wild-type or mutant histamine H_1 receptors were incubated for 180 min at 37 °C (or 25 °C as stated) in 0.5 ml of a 50 mM sodium phosphate buffer (pH 5.8 or 7.4) containing 2 mM EDTA, 3 nM of [^{3}H]mepyramine and increasing concentrations of competing drugs. For saturation binding isotherms, samples were incubated with increasing concentrations of [^{3}H]mepyramine or [^{3}H]levocetirizine (from 0.2 to 20 nM). After the incubation period, receptor bound radioligand was separated from the free ligand by rapid vacuum filtration of the samples over glass fiber filters (type A/C, Pall Life Sciences, VWR international, Leuven, Belgium). Filters were presoaked in 0.1% polyethyleneimine in order to reduce the non specific binding of the radioligand. Adsorbed samples were washed four times with 2 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4). The entire filtration procedure did not exceed 10 s/sample. Radioactivity trapped onto the filter was measured by liquid scintillation counting at 50–60% efficiency.

2.5. Kinetic binding experiments

Association: Binding was initiated by the addition of membranes to the incubation buffer containing 3 nM [^{3}H]mepyramine or [^{3}H]levocetirizine in the presence or absence of cetirizine 10 μM to define non specific binding. At increasing intervals of time thereafter, samples were filtered as described above.

Dissociation: Membranes were added to the incubation buffer containing 3 nM of radioligand and binding was allowed to proceed for 60 or 120 min. At that time, radioligand dissociation was induced by the addition of cetirizine 10 μM. Sample aliquots were taken at increasing time intervals thereafter and filtered as explained above.

To determine the binding kinetics of unlabelled drugs to histamine H_1 receptors, we measured the association